Supercharging of Native-Like Proteins and Protein Complexes: Effects of m-Nitrobenzyl Alcohol versus Sulfolane

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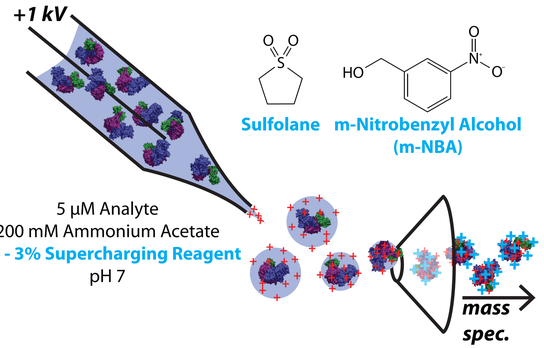
BACKGROUND

Electrospray ionization enables the transport of intact protein and protein complex ions from solution to the gas phase. Typically, electrospray ionization from ammonium acetate buffer solutions produces native mass spectra containing narrow charge-state distributions with low *z*-values. Certain organic molecules, however, can shift the charge-state distribution of electrosprayed proteins to higher *z*-values that are not usually accessible under native conditions. Here, we use sulfolane and m-nitrobenzyl alcohol (m-NBA) to ***supercharge*** proteins and protein complexes.

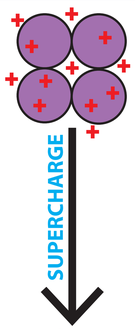


Sulfolane

m-Nitrobenzyl Alcohol (m-NBA)

What are the effects of supercharging on the charge states and structures of proteins and protein complexes?

SUPERCHARGING



Two Conflicting Theories:

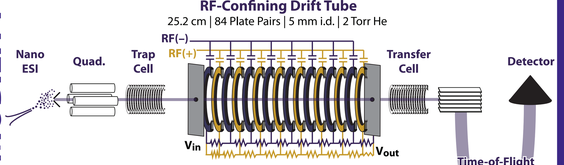
1) ...[supercharged charge states are concomitant with extended, non-native structures]...[1,2]

2) ...[supercharging does not affect native protein structure...any protein instability at high charge states is caused by the interactions of the protein with supercharging reagent]...[3]

	Name	Mass (kDa)
i	cytochrome *c*	12.4
ii	avidin	64.0
iii	bovine serum albumin	67.5
iv	concanavalin A	103
v	alcohol dehydrogenase	148
vi	pyruvate kinase	233
vii	glutamate dehydrogenase	337

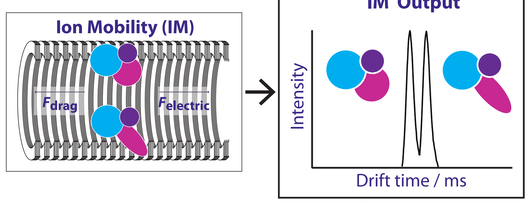
(1) Iavarone and Williams, *J. Am. Chem. Soc.*, **2003**, *125*, 2319-2327.
(2) Sterling, Daly, Feld, Thoren, Kintzer, Krantz and Williams, *J. Am. Soc. Mass. Spectrom.*, **2010**, *21*, 1762-1774.
(3) Lomeli, Yin, Loo and Loo, *J. Am. Soc. Mass Spectrom.*, **2009**, *20*, 593-596.

INSTRUMENTATION & ION MOBILITY



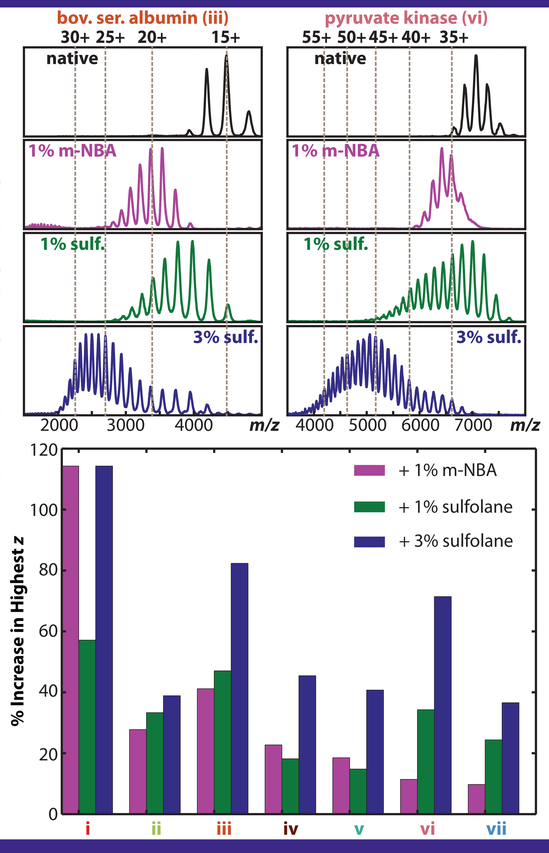
Ions are introduced into our modified Waters Synapt G2 HDMS through nanoelectrospray ionization.

Drift times are measured using an RF-confining drift tube, which contains a stacked ring ion guide that uses alternating RF potentials to radially confine ions and a superimposed DC potential to establish a uniform electric field along the axis of transmission.



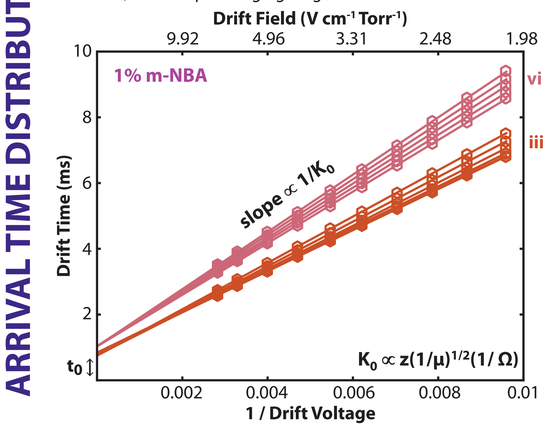
Ion mobility separates ions based on their mobility, which depends on structure and charge. Ions with larger structures will experience more collisions with the neutral background gas and thus have longer drift times than smaller, more compact ions. Different ion conformations result in different drift times.

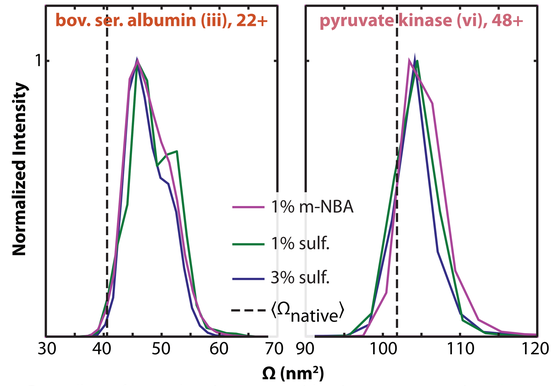
EXTENT OF SUPERCHARGING



ARRIVAL TIME DISTRIBUTIONS

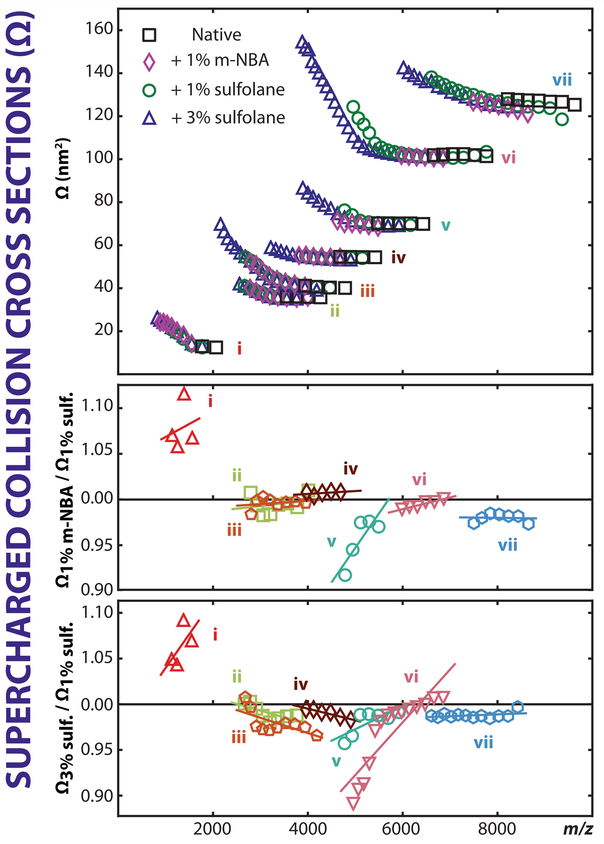
Ion mobility spectrometry was used to probe the collision cross sections (**Ω**) of a large set of native-like protein and protein complex ions generated from solutions containing m-nitrobenzyl alcohol, sulfolane, or no supercharging reagent.





Regardless of supercharging reagent identity or concentration, the supercharged charge states shown above have ***similar structural distributions***.

SUPERCHARGED COLLISION CROSS SECTIONS (Ω)



The two plots above indicate that the **Ω** values of proteins and protein complexes supercharged with either m-NBA or sulfolane are similar for a given charge state. We see that modest supercharging occurs with no change in **Ω**, but that the highest charge states experience the greatest increase in **Ω** and are thus associated with unfolding.

CONCLUSIONS

- A greater percent change in highest *z* was observed for smaller supercharged proteins (<64 kDa). Additionally, 1% m-NBA is a more effective supercharging reagent for smaller proteins, whereas 1-3% sulfolane is more effective for larger proteins (>64 kDa).
- Increases in **Ω** with increasing charge state were much smaller for the largest protein complexes with and without supercharging.
- The results of this study suggest that the extent of supercharging on native protein structure depends strongly on reagent identity, but for most proteins and protein complexes, the **Ω** of a given charge state depends weakly on supercharging reagent identity and concentration.
- Native protein structure can be retained with modest supercharging, however, the highest charge states exhibit varying degrees of unfolding.

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